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RELATION OF THE STRUCTURE OF SUGARS TO THEIR DISSIMILATION IN THE BUTYL-ACETONIC FERMENTATION

JAMES F. GUYMON, L. A. UNDERKOFLER AND ELLIS I. FULMER

The dissimilation of starch in corn mash by *Clostridium acetobutylicum* produces butanol, acetone and ethanol, commonly called "solvents", in the approximate ratio of 60:30:10, respectively. Although corn mash is the usual substrate, fermentations of certain pure carbohydrates by *Cl. acetobutylicum* have been investigated previously to some extent. The studies of various workers (3, 4, 5, 6, 9, 10, 11, 12) have shown that a considerable number of sugars are fermented by the butyl organism in semi-synthetic media. The sugar fermentations are somewhat slower than for corn mash, and the final acidities are somewhat higher with yields of neutral products correspondingly lower. There is some variation in the proportion of solvents produced from the various carbohydrates. Hence, an attempt was made in this investigation to relate the structure of the sugars and the proportions of the solvents formed, by subjecting to the action of the butyl-acetone organism as many of the sugars and polyhydric alcohols as could be readily obtained or prepared. These included thirteen compounds which had not been previously studied in detail, with dextrose and corn mash used for controls.

EXPERIMENTAL

The bacterial culture used was an active culture of the commercial butyl-acetone organism, *Clostridium acetobutylicum*. The methods of conducting the fermentations, the nutrients used in the media, and the analytical methods were as outlined by Underkofler, Christensen and Fulmer (10), except that a modified Shaffer-Somogyi (8) method was employed for sugar determinations. A carbohydrate concentration equivalent in weight of carbon to 4.5 g. of anhydrous dextrose per 100 ml., which in turn is equivalent to 6.25 per cent corn mash in terms of carbohydrate content, was used for all fermentations.

In the experiment now to be described, *d*-galactose, *d*-mannose and lactose, along with dextrose and corn mash as controls, were submitted to the action of the butyl organism for the purpose of following the course of the production of butanol, acetone, and ethanol. In addition, total acidity, pH and amount of carbohydrate remaining were determined. Immediately after inocula-

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tion, and at various intervals thereafter, samples for analysis were removed by means of sterile pipets. The data are assembled in Table I. Mannose was dissimilated almost as completely as dextrose, but somewhat more rapidly, while only about four-fifths of the lactose was fermented and about one-third of the available galactose. At the completion of the lactose fermentations the proportion of butanol was a little higher and of ethanol lower than the normal, while the galactose fermentations gave high acidities and poor yields of neutral products with relatively higher than normal proportion of butanol and low proportions of acetone and ethanol.

A study of the fermentation of *d*-arabinose promised to be of considerable interest in view of the behavior of its optical antipode, *l*-arabinose, which is readily dissimilated by the butyl organism, but which yields a ratio of neutral solvents of 50:40:10 instead of the usual 60:30:10. A quantity of *d*-arabinose was prepared from calcium gluconate by Hockett and Hudson's (1) improvement of the Ruff (7) method. Several attempts to ferment *d*-arabinose in the semi-synthetic medium resulted in failure. Hence, it was decided to employ this sugar in a fermentation series in which varying quantities of corn meal were replaced by equivalent weights of the sugar. Also *l*-rhamnose, *l*-sorbose and *d*-sorbitol, other compounds which had failed to ferment in semi-synthetic media, were employed in similar replacement series along with a dextrose series for control. An amount of corn-gluten meal equal to 1.5 per cent of the volume of medium was added to each flask in order not to seriously deplete the nutrient supply with increasing replacement of corn meal. The results for the experimental series are given in Table II. It is evident that *d*-arabinose, *l*-rhamnose, *l*-sorbose and *d*-sorbitol are not utilized by the butyl-acetone organism.

The pentose sugar, *d*-lyxose, was prepared by the degradation of calcium galactonate with hydrogen peroxide in a manner similar to the preparation of *d*-arabinose from calcium gluconate. Calcium galactonate was made by electrolytic oxidation of galatose according to the method of Isbell and Frush (2). The lyxose was obtained in the form of a sirup which failed to crystallize. The phenylosazone obtained from the sirup had the properties of *d*-lyxosazone (m.p. 162-163° C.). Several attempts to ferment the lyxose sirup in semi-synthetic medium failed. The conclusion that this compound is not fermentable, however, is not fully justified

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since impurities toxic to the butyl organism might possibly have been present in the sirup.

The possible role of dihydroxyacetone as an intermediate in carbohydrate metabolism warranted a study of its fermentability by the butyl organism. When the dihydroxyacetone was added to the medium before sterilization it underwent decomposition during the heating which rendered the medium totally unfermentable. When the dihydroxyacetone was added to sterile semi-synthetic medium at the time of inoculation it was partially fermented; about one-sixth of the compound was utilized as determined by reducing sugar analysis. Unfortunately, insufficient dihydroxyacetone was available to prepare a volume of medium sufficiently large for usual solvents analysis.

Perseitol, a heptahydric alcohol, *i*-inositol, a cyclic hexahydric alcohol, and *l*-arabitol, a pentahydric alcohol, were tested for fermentability by the butyl organism in test tube quantities of semi-synthetic media. None of these compounds was fermented.

Discussion

It has been found, as a result of these studies, together with those of previous investigators, that the only carbohydrates which can be dissimilated by the butyl organism are certain of those which are found naturally occurring. In Table III are given the data for solvents production from the compounds which have been investigated in detail. The fermentability of all of these compounds was checked during the present investigation, but only dextrose, mannose, galactose and lactose were studied in detail. The data for the remaining compounds listed are taken from the papers of Johnson, Peterson and Fred (3), Underkofler, Christensen and Fulmer (10) and Underkofler and Hunter (11).

The butyl-acetonic fermentations of the majority of the various pure carbohydrates which have been studied produce at the end of the fermentations the neutral compounds in relative proportions not greatly different from those obtained in the fermentation of corn mash, i. e., 60 parts butanol, 30 parts acetone and 10 parts ethanol by weight. For some sugars this ratio is practically constant throughout the fermentation, as is also true in the case of corn mash; for others the ratio may change markedly during the course of the fermentation. Most notable examples of such changes in ratio during fermentation are found with the pentoses and the disaccharides.

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Considering only the hexoses and disaccharides listed in Table III, it appears that the carbohydrates which ferment slowly with incomplete dissimilation give lower final proportions of ethanol and higher proportions of butanol than are obtained from corn mash. From the more readily fermented sugars, the solvents ratios are essentially the same as from corn mash, except that there is a tendency for slightly higher proportions of ethanol to be obtained from dextrose, maltose and levulose at the expense of the butanol or acetone.

For the pentoses, data are available only for xylose and *l*-arabinose. Xylose is fermented less rapidly than arabinose. Xylose and arabinose, somewhat like galactose and lactose, give high proportions of ethanol and low percentages of butanol at the beginning of the fermentation. During the fermentations butanol proportions increase in amount, as do also the acetone percentages, while ethanol proportions decrease to the final values. Of all the carbohydrates fermented, only *l*-arabinose has given a proportion of acetone higher than about 30 per cent.

Mannitol, the only polyhydric alcohol fermented, gives a much higher proportion of butanol and lower percentages of acetone and ethanol than are obtained from corn mash, the ratio being 82:12:6. From the beginning of the fermentation the percentages of butanol and ethanol decrease to the final values, while the proportion of acetone increases. The increased percentage of butanol at the expense of acetone is probably due to the fact that mannitol has two more atoms of hydrogen per molecule than do the hexose sugars. Johnson, Peterson and Fred (3) concluded that the fermentation mechanisms for dextrose and mannitol are similar except that the two additional atoms of hydrogen available from mannitol result in increased production of the reduced product, butanol, at the expense of the acetone.

Only a small percentage of all of the possible isomeric hexoses and pentoses and polyhydric alcohols have been submitted to the action of the butyl-acetone organism. And of those tried, only certain of the naturally occurring compounds have proven fermentable. Therefore any conclusion relating sugar configuration to the chemism of the fermentation must of necessity be based upon incomplete information. It is apparent, however, that stereoconfiguration is of prime importance since *l*-arabinose is readily dissimilated while *d*-arabinose is unattacked. Apparently the configuration of a naturally occurring sugar is the favorable

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Table 1. Course of the Butyl-Acetonic Fermentation of Several Carbohydrates

Time hrs.	Acid ml. O. 1 N per 10ml.	Sugar, g./100 ml.	Total Solvents Yield, % of dextrose equiv.	Solvents [A] Proportion, % of total			Acid ml. O. 1 N per 10ml	Sugar, g./100 ml.	Total Solvents Yield, % of dextrose equiv.	Solvents [A] Proportion, % of total		
				B	A	E				B	A	E
	Corn Mash						Dextrose					
0	1.1						1.1	4.50				
8	3.5						2.2	4.33				
12	3.8						2.9	4.15				
16	2.0						3.2	3.97				
20	1.8		17.8	58	30	12	3.4	3.78	3.9	70	24	6
24	1.9						3.5	3.36				
30	1.7		30.9	57	30	13	2.7	2.86	11.7	62	29	9
36	1.9						2.8	1.90				
42	1.9						3.1	0.83				
50	2.0		32.6	58	31	11	2.8	0.27	32.7	56	28	16
60	2.0						3.0	0.19				
72	2.3		32.0	58	30	12	3.1	——	34.5	56	28	16
96	2.4		31.5	58	30	12	3.3	0.13	33.8	57	28	15
126	2.4		32.0	58	30	12	3.4	0.16	34.5	57	28	15
	Mannose						Lactose					
0	1.6	4.52					1.1	4.52				
8	2.9	4.43					2.2	4.46				
12	3.5	4.10					2.8	4.34				
16	4.3	3.91					3.2	4.19				
20	4.3	3.43	6.1	67	28	5	3.5	4.01	3.5	44	30	26
24	4.0	3.09					3.7	3.83				
30	3.2	1.99	18.1	60	30	10	3.7	3.75	7.6	53	32	15
36	3.3	0.79					4.8	3.19				
42	3.8	0.35					2.8	2.67				
50	3.9	0.31	32.3	63	27	10	2.6	2.11	20.5	59	32	9
60	4.2	0.26					2.9	1.35				
72	4.3	——	31.8	62	27	11	2.9	1.03	27.5	62	31	7
96	4.6	0.22	32.8	60	28	12	3.0	0.82	28.2	63	31	6
126	4.4	0.27	33.1	58	30	12	3.0	0.94	28.5	63	31	6
	Galactose											
0	1.4	4.44										
8	2.1	4.20										
12	2.8	4.11										
16	3.3	4.00										
20	3.6	3.96	3.6	57	17	26						
24	3.9	3.83										
30	5.0	3.57	5.0	48	22	30						
36	5.3	3.45										
42	5.6	3.30										
50	6.0	3.22	7.1	56	25	19						
60	6.0	3.19										
72	6.0	3.13	7.9	60	25	15						
96	6.2	2.99	7.5	63	24	13						
126	5.9	2.98	8.5	70	25	5						

[A] B = butanol; A = acetone; E = ethanol.

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Table II. Fermentation of Corn Meal—Carbohydrate Mixtures

Corn Meal Replaced, %	Total Solvents Yield, % of dextrose equivalent				
	Dextrose	*d*-Arabinose	*l*-Rhamnose	*l*-Sorbose	*d*-Sorbitol
0	26.4	26.2	26.1	25.9	27.9
10	25.8	——	——	27.3	21.5
20	25.9	22.1	23.9	20.5	19.9
30	26.1	——	——	20.1	18.5
40	27.2	15.7	17.2	17.9	15.7
50	27.1	——	——	15.3	13.2
60	27.5	7.9	11.6	12.7	10.9
70	28.3	——	——	7.8	6.7
80	28.2	1.9	4.1	4.3	3.6
90	28.1	——	——	1.9	0.6
100	27.9	0.0	0.0	0.4	0.0

Table III. Initial and Final Solvents Ratios for Various Carbohydrates

Carbohydrate	Initial Ratio			Final Ratio			Character of the Fermentation
	B	A	E	B	A	E	
Starch (corn mash)	58	30	12	58	30	12	Rapid and complete
Dextrose	70	24	6	57	28	15	Complete, less rapid than corn mash
Mannose	67	28	5	58	30	12	Complete, more rapid than dextrose
Galactose	57	17	26	70	25	5	Low utilization and sluggish, no acidity break
Lactose	44	30	26	63	31	6	80% utilized, somewhat sluggish
Sucrose	73	26	1	64	32	4	Incomplete utilization, sluggish
Maltose	76	24	0	61	24	15	Rapid and complete
Levulose	63	24	13	58	25	17	Rapid and complete
l-Arabinose	41	33	26	51	39	10	Rapid and complete, high proportion of acetone
Xylose	29	25	46	60	30	10	Less rapid and less complete than arabinose, normal proportion of acetone
Mannitol	88	3	9	82	12	6	Rapid and complete

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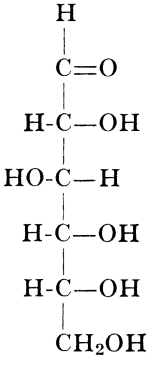
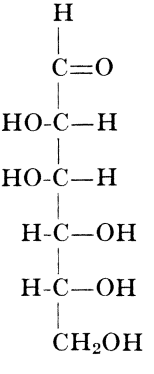
arrangement between two optical antipodes. Most of the sugars which occur in nature belong to the dextro series, *l*-arabinose being an exception.

Speakman (9) based his discussion of the relationship of structure to ease of fermentability upon the presence of adjacent hydroxyl groups in the molecule. On the basis of our results, and dismissing from consideration the influence of optical isomerism, it appears possible that the position of a pair of adjacent hydroxyl groups with respect to the terminal carbon position is significant. The structural formulae of the compounds under consideration are given below:

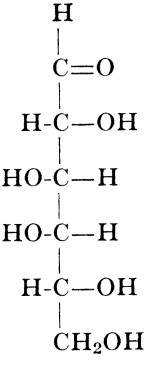
ALDO-HEXOSES



d-Glucose (dextrose)

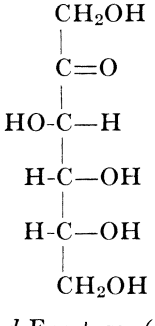


d-Mannose

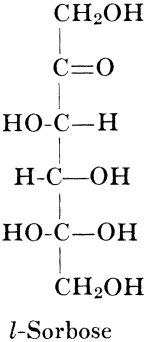


d-Galactose

KETO-HEXOSES

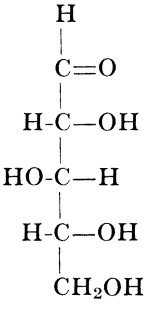


d-Fructose (levulose)

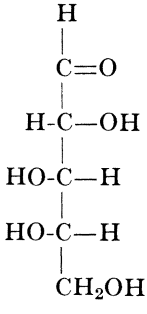


l-Sorbose

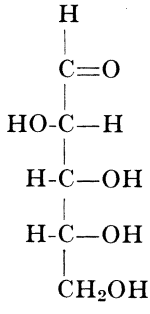
Aldo-Pentoses



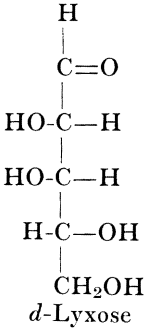
d-Xylose



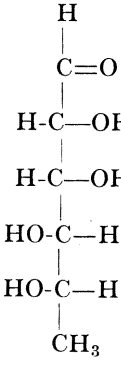
l-Arabinose



d-Arabinose

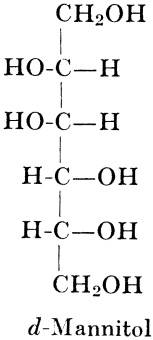


d-Lyxose

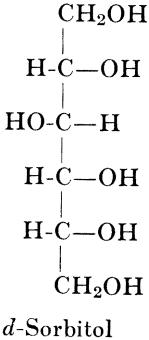


l-Rhamose (methyl pentose)

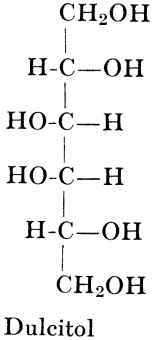
Polyhydric Alcohols



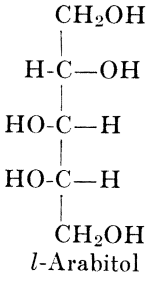
d-Mannitol



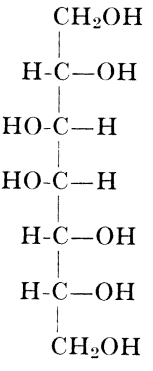
d-Sorbitol



Dulcitol



l-Arabitol



Perseitol (*d*-manno *d*-gala-heptitol)

Judging from the behavior of the sugars whose structures are shown, the configuration of a sugar most favorable toward fermentability by the butyl organism requires a pair of *cis* hydroxyl groups located in the molecule adjacent to a primary alcohol group. For the aldo-hexoses both mannose and glucose ferment well and possess such an arrangement, whie galactose, which is poorly fermented, does not. The latter sugar contains one pair of *cis* hydroxyls but this pair is centrally situated. The theory holds for the two keto-hexoses studied, namely fructose and sorbose. Fructose, possessing a pair of adjacent hydroxyls in the terminal position, is readily fermented; sorbose, having no pair of adjacent hydroxyl groups, is not fermented.

Of the pentoses, only *d*-xylose and *l*-arabinose have been found fermentable. The contrasting behavior in regard to the final solvents ratios from these two sugars has been mentioned. *l*-Arabinose, which is fermented somewhat more readily than xylose, contains a pair of adjacent hydroxyl groups in the terminal location opposite the carbonyl group, while xylose possesses no adjacent pair. *d*-Lyxose contains a pair of *cis* hydroxyls but not situated in the terminal position. It is not a naturally occurring sugar and is apparently not fermented. The methyl pentose, *l*-rhamnose, occurs in nature and has a structure similar to mannose in regard to pairs of adjacent hydroxyl groups, but its inability to be utilized by the butyl organism is probably associated with the presence of the methyl group instead of the primary alcohol group.

d-Mannitol is the only polyhydric alcohol which has been found definitely to be utilizible by the butyl organism. It exhibits a

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favorable configuration in that there are present two pairs of *cis* hydroxyl groups, both in terminal positions. The polyhydric alcohols, dulcitol, *d*-sorbitol, perseitol and *l*-arabitol, occur in nature and each contains pairs of *cis* hydroxyl groups, the latter three compounds having a pair in the terminal position, yet none of these substances have been found to be fermentable. Some other unfavorable condition must predominate in these polyhydric alcohols since none are attacked by the butyl-acetone organism.

It had been hoped that this study, on the basis of sugar structure, would throw some light on the reason for the unusual proportions of solvents formed from certain of the sugars, particularly from *l*-arabinose, the only sugar which gives an unusually high proportion of acetone, as well as from galactose and lactose which give high butanol percentages at the expense of the ethanol. However, the attainment of this goal was prevented by the failure of the organism to ferment any of the synthetic compounds tried along with many of the natural ones. Considering the difficulties in obtaining quantities of the less common sugars sufficient to carry out complete studies of their fermentability, a final satisfactory solution to the question of the influence of configuration on fermentability will necessarily be slow. However, the experimental data now available are sufficient to show a general trend in the relation between configuration and fermentability as discussed above.

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